Reference: *Biol. Bull.* **163:** 131–143. (August, 1982)

THE ANATOMY AND FINE STRUCTURE OF THE EYE IN FISH. VI CILIARY TYPE TISSUE IN NINE SPECIES OF TELEOSTS

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Abstract

The eyes of teleost fishes do not have ciliary bodies. Therefore there is no ciliary epithelium *per se*, the tissue normally assumed to secrete aqueous humor. When examined at the electron microscope level a layer of nonpigmented cells on the back of the fish iris shows many similarities to the ciliary epithelium of mammals. The tissue of fish iris has strategically located zonulae occludents similar to those forming the blood-aqueous barrier in mammals. There is a marked lateral interdigitation of cells as seen in mammalian ciliary tissue and as seen in the specific salt absorbing cells found in the gills of brackish water adapted crabs. The teleost tissue also has numerous intercellular spaces (ciliary channels?) distributed in the same fashion as in mammalian ciliary epithelium. Although there is no morphological evidence for the secretion of aqueous humor, there is indirect evidence that the nonpigmented cells absorb salt to produce the hypotonic aqueous humor that is unique to teleosts.

Introduction

The morphology of the cells or tissue which secretes aqueous humor in the cold blooded vertebrates has received comparatively little attention. Fish have been almost completely neglected. They present an interesting problem in that their eyes completely lack the ciliary muscle and associated epithelium. Instead, the lens is suspended by a membrane dorsally and anchored ventrally by a hillock of muscle (campanula of Halleri). The muscle retracts the lens to accomplish accommodation.

Zadunaisky (1972, 1973) has studied the electrolyte content of the aqueous humor in several fishes and has also made preliminary observations on the possible site of secretory origin. The epithelium on the posterior surface of the iris proves to be the likely source of the primary aqueous humor.

There is universal agreement that in the higher vertebrates the ciliary body in some manner secretes the aqueous humor. The secreted fluid then passes through the pupil to the anterior chamber and exits via the trabecular meshwork and the canal of Schlemm. Evidence indicates that the secretion is accomplished by active transport.

The fine structure of the ciliary body and its epithelium in mammals has been thoroughly investigated. To name a few investigators: Pappas and Smelser (1961); Pappas and Tennyson (1962); Tormey (1963, 1964); Kaye and Pappas (1965); Bairati and Orzalesi (1966); Smith (1971); Raviola (1971, 1974); Shabo and Maxwell (1972, 1973); Uusitalo *et al.* (1973); Okisaka (1976a, b); Hara *et al.* (1977).

For reviews of the comparative composition of aqueous humor and the release of aqueous humor see Cole (1974), and Tripathi (1974), respectively.

Received 14 August 1981; accepted 12 April 1982.

Abbreviations: NPL, nonpigmented layer; PL, pigmented layer.

The following report is an expansion of Zadunaisky's initial studies and utilizes a wider range of species and different electron microscopy techniques. It also is a continuation of my own investigations of the eyes of fish (Copeland, 1974a,b, 1976, 1980; Copeland and Fitzjarrell, 1975; Copeland and Brown, 1976).

Materials and Methods

The fine structure of the tissues on the back surface of the iris was investigated in a wide variety of available fishes. The following nine species were studied in detail: blue gill, *Lepomis macrochirus* Rafinesque; eel, *Anguilla rostrata* (Lesueur); mummichog, *Fundulus heteroclitus* (Linnaeus); goldfish, *Carassius auratus* (Linnaeus); scup, *Stenotomous chrysops* (Linnaeus); sea horse, *Hippocampus erectus* Perry; three spined stickelback, *Gasterosteus aculeatus* Linnaeus; rainbow smelt, *Osmerus mordax* (Mitchill); rainbow trout, *Salmo gairdneri* Richardson.

The fish were narcotized with Finquel (Ayerst brand of tricaine methane surfonate). If the eye was difficult to enucleate, a window was cut in the cornea and fixative introduced by blunt hypodermic needle to the pupil in a manner to gently flush the back of the iris. The eye was then dissected out of the socket and a circumferential cut made to free the cornea plus part of the sclera and retina, which was then immersed in fixative.

If the eye was large and easily removed, the circumferential cut was made immediately and the front part of the eye immersed then in the fixative.

The fixative was 3% glutaraldehyde together with 1.5% polyoxymethylene (paraformaldehyde) plus 3% sucrose in 0.1 *M* cacodylate buffer adjusted to pH 7.4. Fixation was initiated at room temperature but as soon as the dissections were completed the vials were placed in a refrigerator for six hours. Final trimming was done in cold 0.1 *M* buffer and the tissues left in cold buffer several hours or overnight. Post fixation was done with cold 1% osmium tetroxide in 0.1 *M* cacodylate for 45 minutes. The vials were then brought to room temperature and after several buffer rinses the tissues were stained en bloc with 2% uranyl acetate in 30% acetone. Dehydration was completed in acetone (Baker's Anhydrous 6-A137) and embedment done in Epon 812. Sections were stained with lead citrate.

One of the fixative variations was the use of tannic acid in the first buffer rinse following the primary fixation in an effort to enhance the staining of the tissues (Simionescu and Simionescu, 1976). Results were poor (probably inadequate penetration) except for one fortuitous and unexpected result (see text and Figs. 5 and 10).

Results

The fine structure of the tissue covering the back of the iris in teleost fishes was examined. The tissue is in the form of a single cell layered nonpigmented epithelium (NPL) backed by a layer of pigmented cells (PL). The two layers extend from the retina to the edge of the pupil and in turn are backed by a layer of connective tissue containing blood vessels (Fig. 1).

The most noticeable and consistent characteristic of the NPL is the baso-lateral interdigitations of the cells. (Note: During ontogeny the NPL is folded inward to cover the PL. Thus, the basal surface of the NPL becomes the free surface in the adult eye). The flattened, leaf-like extensions communicate to the surface of the epithelium and reach to varying depths within a neighboring cell. The cells mutually interdigitate on a one-to-one basis. The occasional occurrence of neighboring light and dark cells demonstrates this clearly (Fig. 4).

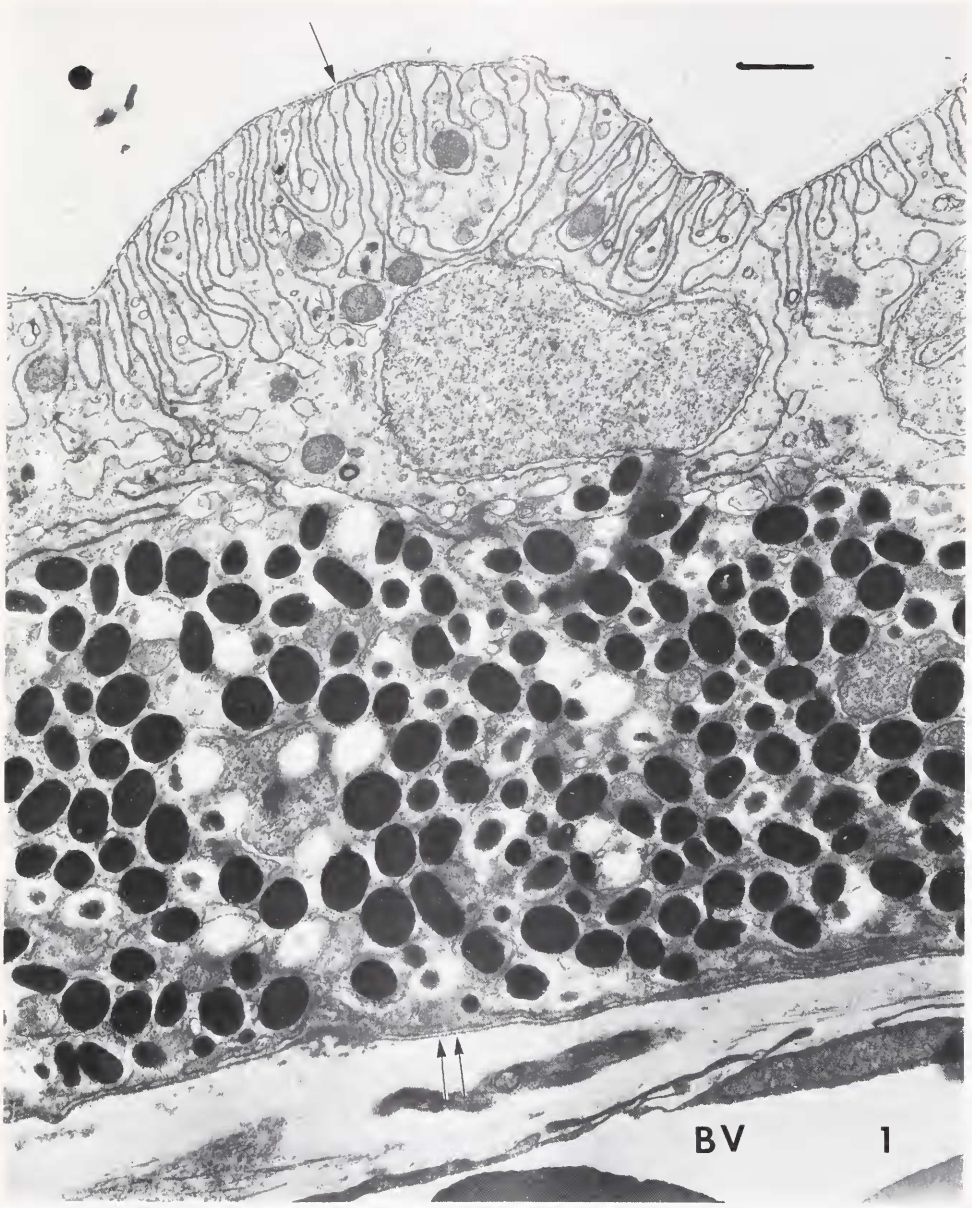

FIGURE 1. *Stickelback.* Low power of both the NPL and PL. The cell in the NPL is covered by an inner limiting membrane (arrow) and shows numerous interdigitating projections from a neighboring cell. The PL cell has a basal lamina (double arrow) and is subtended by a blood vessel (BV). Scale: 1.0 micron.

The inner, vitreal NPL bears an interesting relationship to the subtending PL. Peripherally (*i.e.*, toward the retina) the NPL is devoid of melanin granules. However, as the pupil is approached there is an increasing number of melanin granules to be found in the NPL until at the pupil the two layers are hard to distinguish.

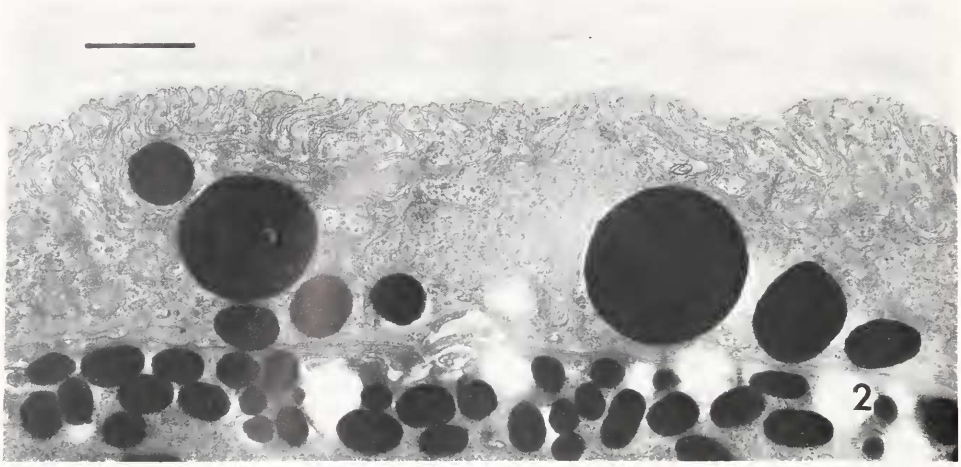

FIGURE 2. *Smelt*. Showing exceptionally large melanin granules in the NPL. Scale: 1.0 micron.

In some instances the granules found in the NPL enlarge enormously (Fig. 2) and in still others they fragment (Fig. 3).

A much less constant characteristic is the nature of the free surface of the NPL. Most of the species examined exhibit a relatively smooth surface. However, goldfish

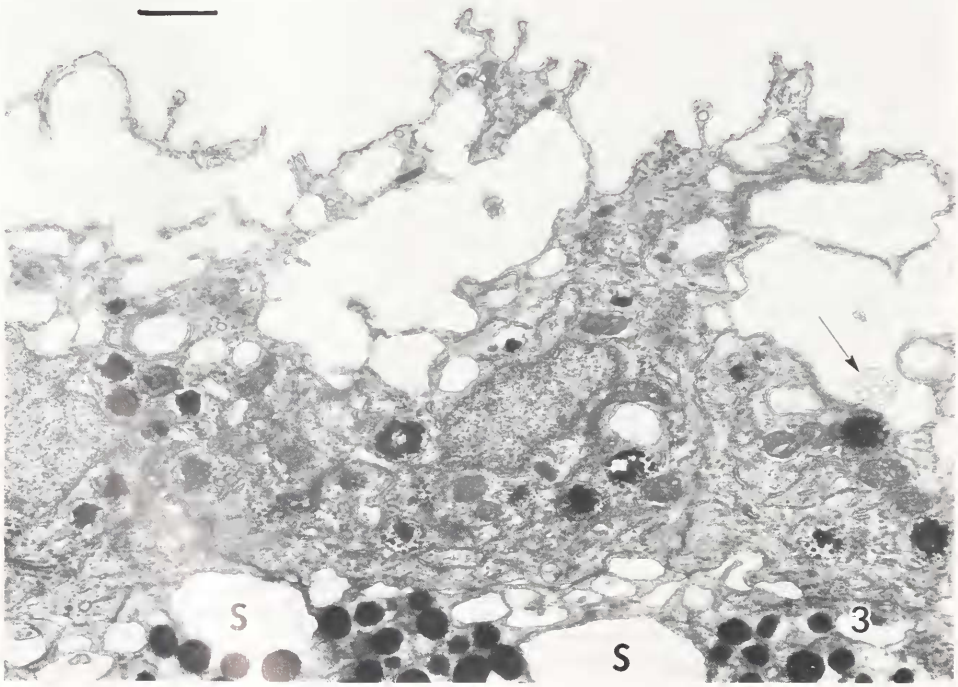

FIGURE 3. *Goldfish*. Several large intercellular spaces (S) are seen between the NPL and PL (ciliary channels?). Multivesiculate body (arrow). Note fragmentation of some of the melanin granules in the NPL. Scale: 1.0 micron.

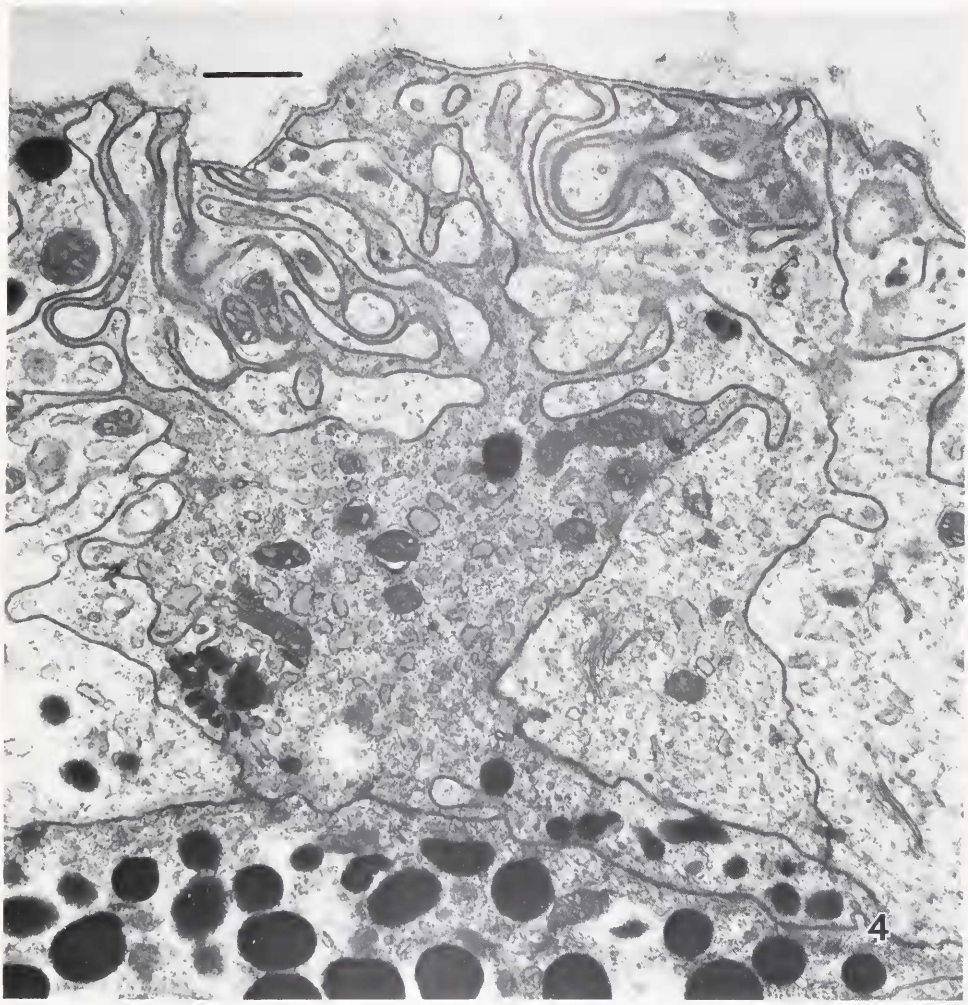

FIGURE 4. *Mummichog.* Low power view of a dark cell with contrasting interdigitations from neighboring light cell(s) of the NPL. Note the one-to-one relationship of the interdigitations. Scale: 1.0 micron.

(Fig. 3), blue gill, and trout have highly irregular, branching folds projecting from the surfaces.

Another characteristic showing a degree of variation is found in the inner limiting membrane on the surface of the epithelium. In most fish it is well developed and strongly adherent (goldfish, Fig. 5 and eel, Fig. 6). In a few it is more fragile and easily lost during the preparative procedures (sea horse, Fig. 7). Due to the happenstance of embryology of the eye, mentioned above, the membrane indeed is a basal lamina.

The mitochondria of all the cells are randomly distributed and showed no preferential orientation to the interdigitated projections. However, they are included sometimes within the more blunt ones (Fig. 1).

Well-developed Golgi apparati and associated membranous structures are located in the scleral end of the NPL. Secretory granules may be seen in the same region (Fig. 8).

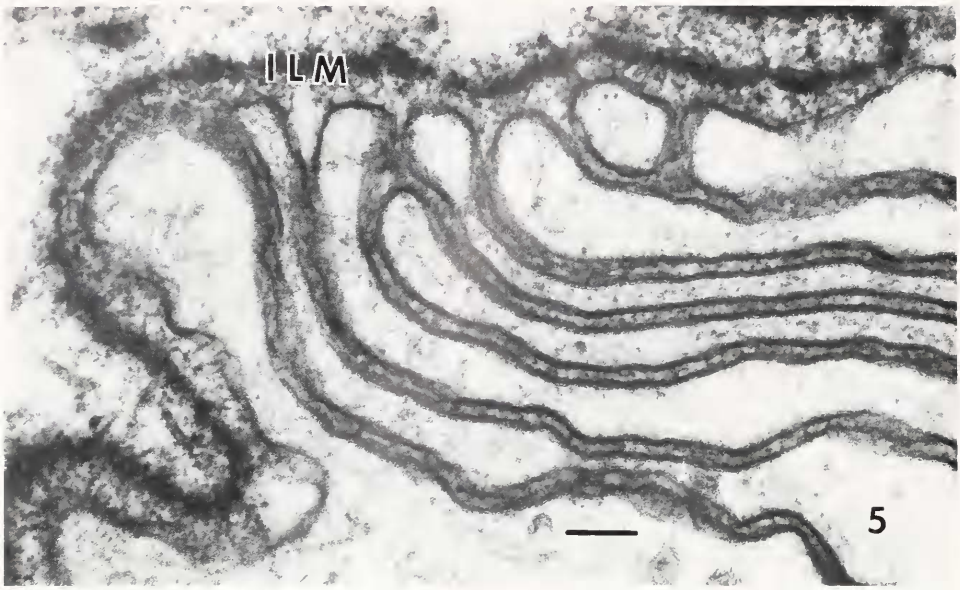

FIGURE 5. *Goldfish.* Surface of the NPL. The inner limiting membrane (ILM) is well developed and adherent. Penetration of tannic acid mordant delineates the intercellular spaces of the interdigitations and the granular material within the spaces. Note there are no cellular junctions. Scale: 0.1 micron.

Although not preserved in all preparations, coated vesicles are seen frequently (Fig. 9). Usually, they are found associated with the free surfaces of the cells.

The NPL has both rough endoplasmic reticulum (usually in the Golgi area) and smooth endoplasmic reticulum throughout the cell.

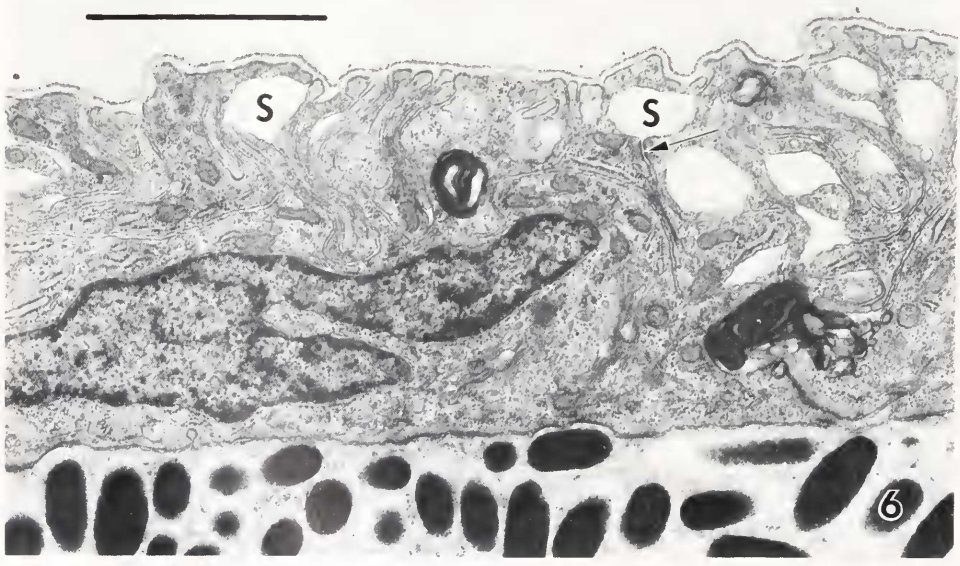

FIGURE 6. *Eel.* Inner limiting membrane well developed. Intercellular spaces are seen (S). A macula type junction or desmosome (arrow) common to the cell body plasma membranes (not to the interdigitations) is seen adjacent to one of the spaces. Scale: 0.1 micron.

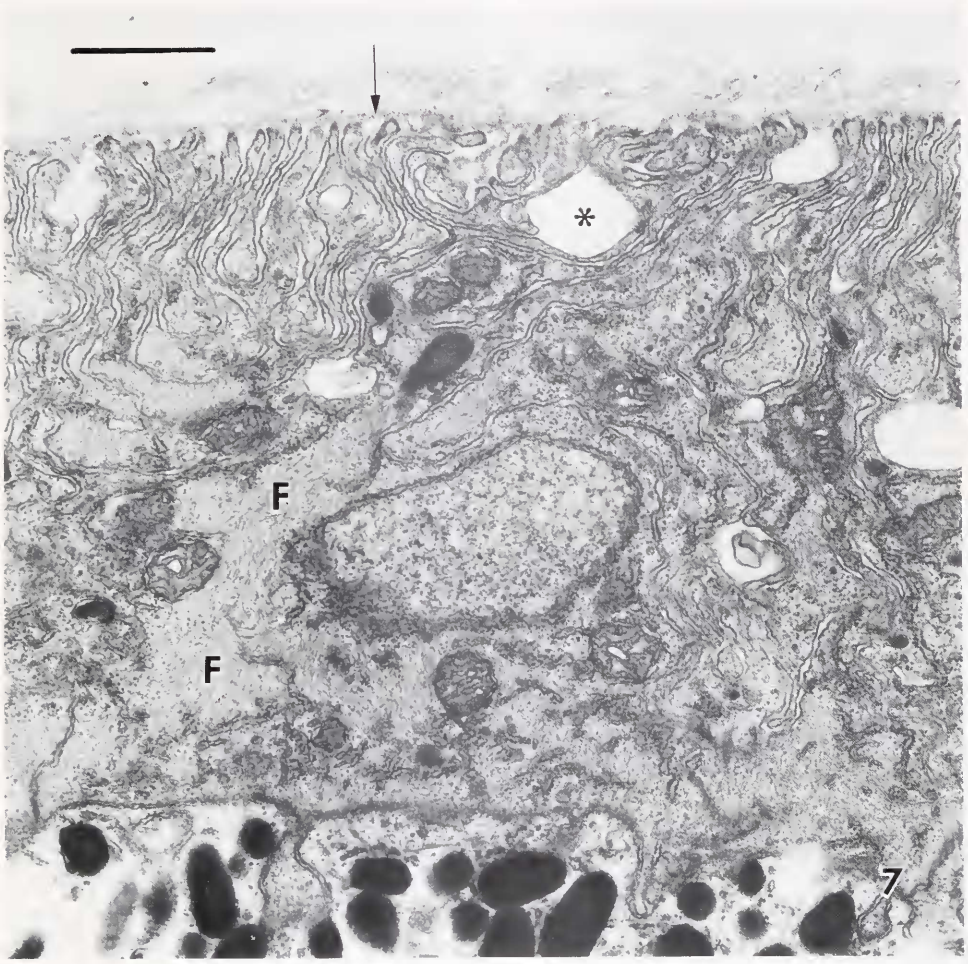

FIGURE 7. *Sea Horse.* A weakly organized inner limiting membrane (arrow) rests on the interdigitations and a few fibers common to the vitreous humor are above the membrane. A high concentration of filaments (F) is present within the NPL cell. A well-developed intercellular space (asterisk) is seen near the surface. Scale: 1.0 micron.

Microtubules are seen occasionally in the cell surface areas, but much more predominant are clusters of small fibrils (Fig. 10). In one fish, sea horse, they occupy a good share of the cell cytoplasm (Fig. 7). They are of the order of intermediate or 10 nm filaments. That is, they are "intermediate" to microtubules at 24 nm and microfilaments at 5–7 nm.

The space between the interdigitating plasma membranes of the adjoining cells of the NPL is open to the free surface. Due to a fortuitous usage of tannic acid technique, in one instance the plasma membranes are not only selectively stained but particulate material is seen in the intercellular space (Figs. 5 and 10). The same type of particles seen beneath the inner limiting membrane is found also between the cells (Fig. 5). Desmosomes are found at random intervals between the plasma membranes of the cell bodies proper, but are seen rarely between the membranes of the complementary interdigitations.

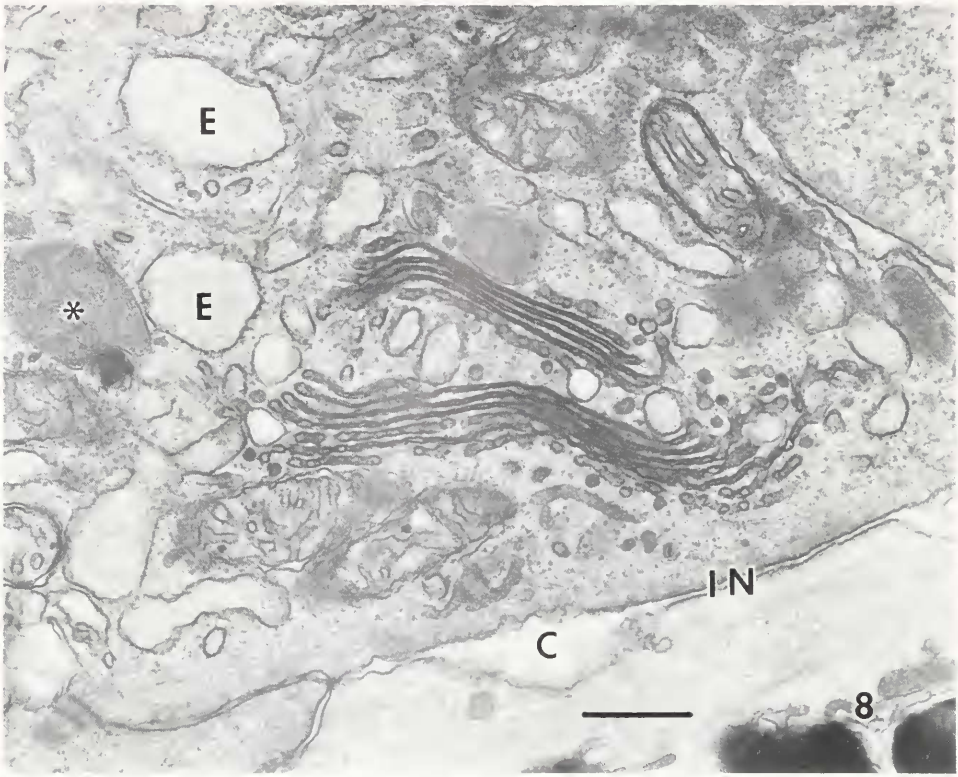

FIGURE 8. *Scup.* Typical Golgi apparatus within the NPL and adjacent to the PL interface (IN). Expanded endoplasmic reticulum (E) with granular material. Secretory granule (asterisk). Ciliary channel (C). Scale: 1.0 micron.

The size and number of intercellular spaces in the NPL varies from species to species. They may be almost nonexistent, as in stickelback (Fig. 1), smelt, scup, mummichog; small, eel (Fig. 6) and sea horse (Fig. 7); large, goldfish (Fig. 3) and blue gill; or very large, trout. In some cases, spaces are seen also between the NPL and PL (Fig. 8). The spaces frequently contain fine granular material and, at times, membranous, multivesiculate material.

The PL shows little activity compared to that seen in mammalian species. The cells are filled with melanin granules and have only a few structures such as mitochondria, Golgi apparati, endoplasmic reticulum, *etc.* Occasionally, a mild degree of interdigitation occurs between the end of the cells facing the vascular vessels. There also are occasional intercellular spaces filled with granular material similar to that seen in the spaces of the NPL.

DISCUSSION

My observations on the fine structure of the goldfish NPL are not in complete agreement with Zadunaisky's description of the same species. His "microvilli" are in reality tortuous folds or outpocketings of the cell surface. Also he did not note the cellular interdigitations to be found in the NPL of the fish that he describes (goldfish). The interdigitations may not be as numerous or complex as in other fish, but they are present.

The interdigitation of the neighboring cells is the most consistent feature common to all the fish studied. The dimensions of the interdigitations vary somewhat from species to species but that is not an artifact (*i.e.*, exactly the same fixative procedures were used throughout). The differences may be due to slight differences in the tonicity and/or ionic balance in the respective aqueous humors, factors not known at present.

Also, noteworthy is the fact that a plicated or ruffled surface of the NPL is found only in three fresh water forms (goldfish, blue gill and trout). The surfaces are smooth in the six sea water species (and in a number of other sea water fishes not described here). Although suggestive of a true difference beween fresh water and sea water fish, a greater number of fresh water fish would need to be examined to determine the validity of such an indication.

The fine structure of the epithelium on the back of the teleost iris bears a close and striking resemblance to the ciliary epithelium of the mammals.

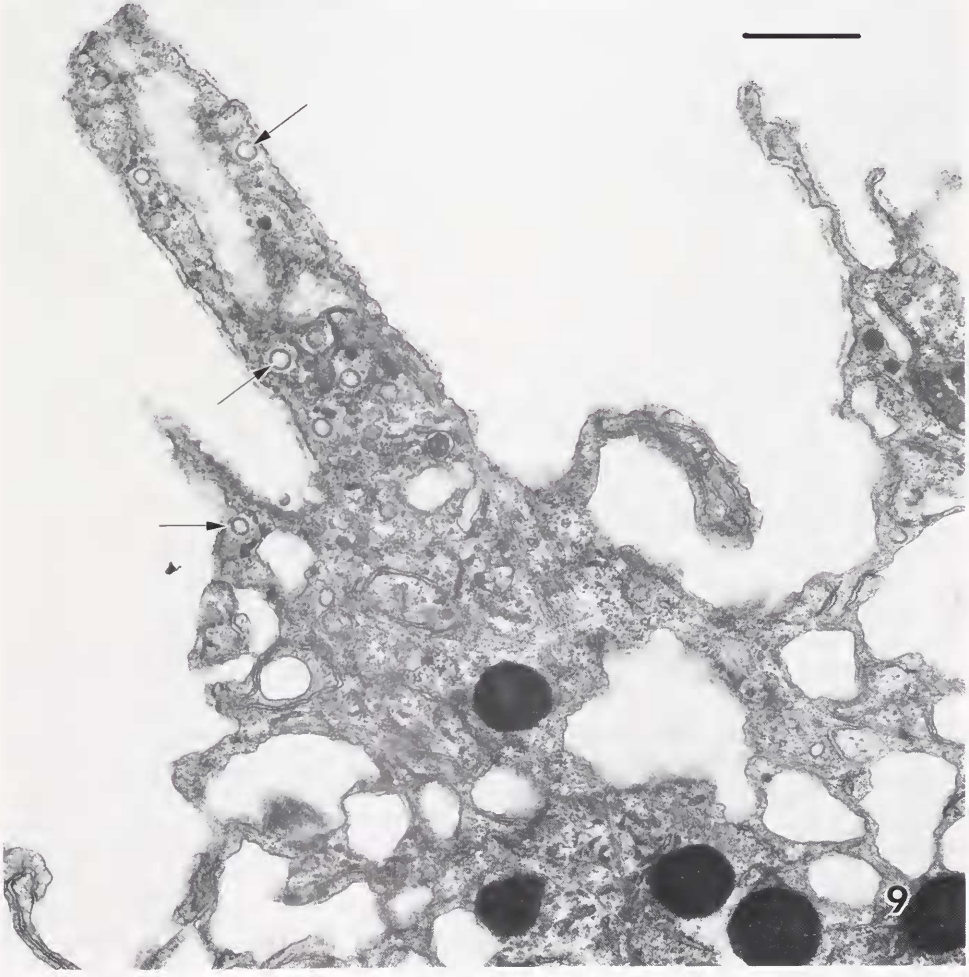

FIGURE 9. *Goldfish.* Tip of one of the surface ruffles showing coated vesicles (arrow). Scale: 1.0 micron.

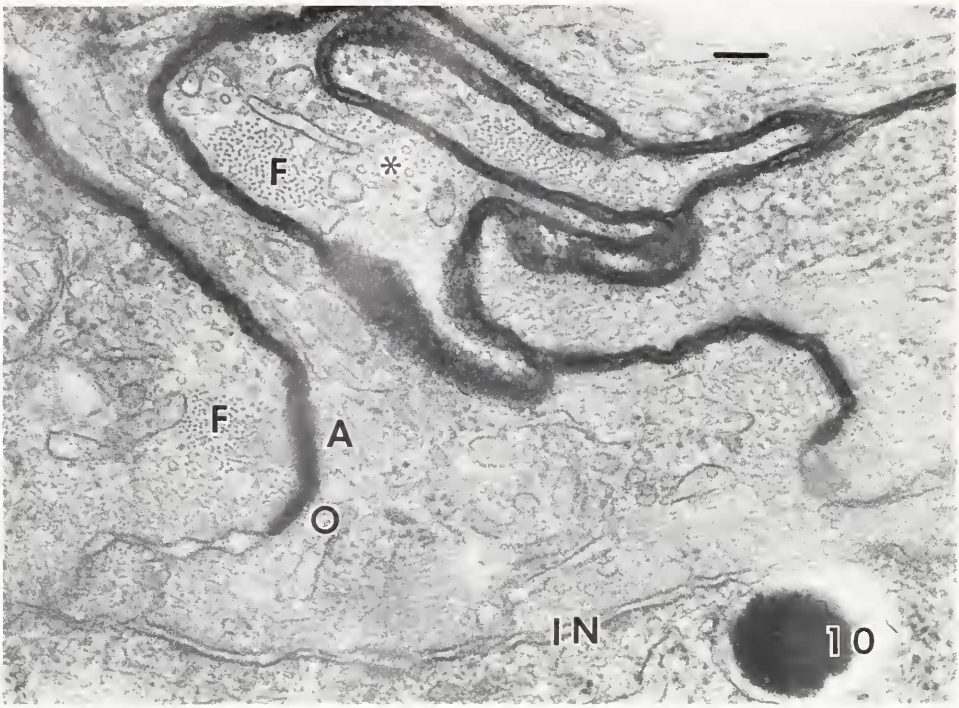

FIGURE 10. *Goldfish.* NPL at its interface (IN) with the PL. From same tissue block as in Figure 5 but at a lower power. Note that the penetration of tannic acid into the intercellular space is limited by the zonula occludente (O). Cross section of intermediate filaments (F). A few microtubules (asterisk). Zonula adherente (A). Scale: 0.1 micron.

Highly noteworthy is the existence of cellular interdigitations in the NPL of fish. These are of a type and orientation similar to those seen in mammals (references listed in the Introduction). The interdigitation in teleosts is on a one-to-one basis as demonstrated by the fortuitous association of light and dark cells. Tormey (1963, 1964) and Kaye and Pappas (1965) made the same type of observations in the rabbit ciliary epithelium.

Equally significant, the barrier of zonulae occludentes and associated zonulae adherentes found at the apex of the NPL of mammals (Bairati and Orzalesi, 1966; Shabo and Maxwell, 1972; Uusitalo *et al.*, 1973; Raviola, 1974; Okisaka, 1976b) is found also in fish. Though none of the usual tracers were used in the present investigation, the happenstance of limited tannic acid penetration between plasma membranes validates this interpretation.

The NPL shows all the fine structure usually seen in secretory cells. There is a plentiful supply of membranous organelles such as Golgi apparati, endoplasmic reticulum (rough and smooth), mitochondria, and granules filled with particulate material.

The PL is packed with the melanin granules and shows almost none of the morphology usually associated with metabolic or secretory activity. The basal-lateral surfaces of the cells show a mild degree of interdigitation but in no way approach the complexity seen, for example, in the monkey (Okisaka, 1976a).

One of the prime "road blocks" found in the current literature is the commonly held belief that the zonulae occludentes junctions in the NPL represent an inviolate

blood-aqueous barrier. The work of Raviola (1974) gives most excellent support to this idea. Nevertheless, it should be kept in mind that living cells are dynamic systems and they could well eliminate and reform junctions as they do other organelles. It is of puzzling significance that the intercellular spaces between the NPL and PL (and frequently within the PL) have the same appearing content (multivesiculate or granular) as the intercellular spaces in the NPL. The finely granular material is seen consistently enough to suggest that it is a normally occurring material. However, the multivesiculate type clusters, also sometimes seen in the spaces, occur randomly enough that they could be artifacts.

If, as repeatedly stated in the literature, there is indeed an inviolate blood-aqueous barrier in the distal borders of the NPL by reason of zonulae occuludentes, then attention must be turned to the basal interdigitations where only local, maculae type junctions occur infrequently. Here interpretation of function, though indirect, can be made more plausible as explained below.

Zadunaisky (1972) has shown that the aqueous humor in two teleost fishes (goldfish and the marine sargus) is hypotonic to the blood plasma, contrary to the situation in mammals and amphibia. His experimental physiological procedures indicated that sodium and potassium are preferentially absorbed to effect the lowered tonicity. Later, at the fine structure level he found a histochemical localization of ATPase on the free surface of the NPL (Zadunaisky, 1973). He interprets this as a possible site for the metabolic pump that could account for the absorption of electrolytes during the formation of the *hypotonic* aqueous of the fish eye. It is of related significance that Kaye and Pappas (1965) found ATPase on the free surfaces and interdigitations of the equivalent tissue in the rabbit. They, however, interpret the presence of the enzyme as facilitating the secretion of electrolytes in the formation of the *hypertonic* aqueous of the rabbit eye.

There already exists an excellent example of a one-to-one interdigitation in a tissue whose function is specifically osmoregulatory. The marine blue crab, *Callinectes sapidus*, invades very dilute marsh areas in the warmer months in search of food and are found in waters with as low as 0.5‰ total salinity. Salt is then absorbed through an epithelium that lines a part of the vascular space of the gills. Physiological proof of salt absorption by crab gills was provided by Nagel (1934) and Koch *et al* (1954). The fine structure of the single cell layered epithelium has been studied by Copeland and Fitzjarrell (1968). The cells laterally interdigitate quite deeply on a one-to-one basis (see Fig. 7, page 8, Copeland and Fitzjarrell, 1968).

The striking similarity between the crab gill tissue, which is specifically devoted to salt transport, and the NPL of fish and mammals is remarkable. This morphological coincidence plus the physiological determinations made by Zadunaisky (1963) suggest that the NPL has an osmoregulatory function related to the ultimate producton of aqueous humor.

Thus, at the morphological level, two functions can be suggested for the combined NPL and PL of the fish iris. One, the presence of similar intercellular granular material in both the layers suggest a secretion of aqueous humor precursor by way of the spaces between the cell bodies. Two, the plicating interdigitations of the NPL may refine the aqueous humor by means of absorbing specific electrolytes.

Acknowledgment

This work was supported by HEW-NEI grant number EY-02647.

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